

AMENDMENT TO THE CLAIMS

This listing of claims replaces all prior versions of claims in the application

Listing of claims

1-107 (Cancelled)

108. (New) An expression construct encoding an RNA complex comprising a first strand and a second strand that are capable of hybridizing to each other under physiological conditions to form a double-stranded region, said double-stranded region comprising one or more mismatched regions that separate said double-stranded region into two or more double-stranded segments, wherein said mismatched regions are susceptible to cleavage by single-strand ribonucleases and wherein at least a portion of at least one of said double-stranded segments has substantial sequence identity to a target polynucleotide sequence, or

an RNA molecule comprising one or more stem-loop structures comprising a double-stranded stem region and a single-stranded loop region each separated by a single-stranded spacer region, wherein the double-stranded stem region of at least one stem-loop structure comprises one or more mismatched regions, wherein said one or more mismatched regions separates said double stranded stem region into two or more double stranded segments, wherein said mismatched regions are susceptible to cleavage by single-strand ribonucleases and wherein at least a portion of at least one of said double-stranded segments has substantial sequence identity to a target polynucleotide sequence.

109. (New) The expression construct of claim 108, wherein said expression construct encodes said RNA complex.

110. (New) The expression construct of claim 108, wherein said expression construct encodes said RNA molecule.

111. (New) The expression construct of claim 108, wherein said expression construct is a deoxyribonucleic acid (DNA) molecule which is capable of expressing said RNA complex or RNA molecule.

112. (New) The expression construct of claim 108, wherein said first and second strands of said RNA complex are joined.

113. (New) The expression construct of claim 112, wherein said first and second strands of said RNA complex are joined by a loop region.

114. (New) The expression construct of claim 108, wherein said expression construct further encodes an RNA hairpin comprising, in 5' to 3' order, an A strand and a B strand, said A and B strands capable of hybridizing under physiological conditions and

said A and B strands joined by a loop region, wherein, upon expression of said RNA complex, the 3' end of said B strand is joined to the 5' end of said RNA complex or said RNA molecule by a C strand, and wherein said hairpin stabilizes said RNA complex or said RNA molecule, relative to an RNA complex or RNA molecule lacking said hairpin.

115. (New) The expression construct of claim 114, wherein said hairpin is a Bernie Moss (BM) hairpin.

116. (New) The expression construct of claim 108, wherein at least a portion of at least one of said double-stranded segments of said RNA complex or said RNA molecule has substantial sequence identity to said target polynucleotide sequence, and wherein said RNA complex or said RNA molecule is capable of reducing expression of said target polynucleotide sequence, relative to expression of said target polynucleotide sequence in the absence of said RNA complex or said RNA molecule.

117. (New) The expression construct of claim 116, wherein said RNA complex or RNA molecule comprises two or more double stranded segments having substantial sequence identity to the same target polynucleotide sequence.

118. (New) The expression construct of claim 116, wherein said RNA complex or

RNA molecule comprises two or more double stranded segments having substantial sequence identity to different target polynucleotide sequences.

119. (New) The expression construct of claim 116, wherein said RNA complex or RNA molecule comprises two or more double stranded segments having substantial sequence identity to different polynucleotide sequences within the same target polynucleotide sequence.

120. (New) The expression construct of claim 116, wherein said RNA complex or RNA molecule comprises two or more double stranded segments having substantial sequence identity to different polynucleotide sequences within different target polynucleotide sequences.

121. (New) The expression construct of claim 116, wherein said RNA complex or said RNA molecule comprises a plurality of double-stranded segments each of which has substantial sequence identity to a target polynucleotide sequence.

122. (New) The expression construct of claim 108, wherein said RNA complex or said RNA molecule comprises at least two mismatched regions that separate said double-stranded region of said RNA complex or at least one double-stranded stem region of said

RNA molecule into at least three double-stranded segments.

123. (New) The expression construct of claim 108, wherein one or more of said double-stranded segments has at least 18 contiguous nucleotides with substantial sequence identity to said target polynucleotide sequence.

124. (New) The expression construct of claim 123, wherein one or more of said double-stranded segments comprises 19 to 30 contiguous nucleotides with substantial sequence identity to said target polynucleotide sequence.

125. (New) The expression construct of claim 108, wherein the first or second strand of said RNA complex comprises a 5' or a 3' end having a single-strand overhang comprising at least one nucleotide, wherein said nucleotide does not base-pair with another nucleotide.

126. (New) The expression construct of claim 108, wherein said RNA molecule comprises a 5' or a 3' end having a single-strand overhang comprising at least one nucleotide, wherein said nucleotide does not base-pair with another nucleotide.

127. (New) The expression construct of claim 113, wherein at least one of said

loop region of said RNA complex comprises 4 to 10 nucleotides that do not base-pair with a nucleotide of said RNA complex.

128. (New) The expression construct of claim 108, wherein each of said one or more mismatched regions comprises at least 2 nucleotides.

129. (New) The expression construct of claim 113, wherein said RNA complex further comprises, at the 5' or 3' end of said first or second strand, one or more stem-loop structures comprising a double-stranded stem region and a single-stranded loop region each separated by a single-stranded spacer region, wherein the double-stranded stem region of at least one stem-loop structure comprises one or more mismatched regions, wherein said one or more mismatched regions separates said double stranded stem region into two or more double stranded segments, wherein said mismatched regions are susceptible to cleavage by single-strand ribonucleases and wherein at least a portion of at least one of said double-stranded segments has substantial sequence identity to a target polynucleotide sequences.

130. (New) The expression construct of claim 129, wherein two or more double-stranded segments within the stem-loop structures of said RNA complex have substantial sequence identity to the same target polynucleotide sequence.

131. (New) The expression construct of claim 129, wherein two or more double-stranded segments within the stem-loop structures of said RNA complex have substantial sequence identity to different target polynucleotide sequences.

132. (New) The expression construct of claim 129, wherein two or more double-stranded segments within the stem-loop structures of said RNA complex have substantial sequence identity to different polynucleotide sequences within the same target polynucleotide sequence.

133. (New) The expression construct of claim 129, wherein two or more double-stranded segments within the stem-loop structures of said RNA complex have substantial sequence identity to different polynucleotide sequences within different target polynucleotide sequences.

134. (New) The expression construct of claim 129, wherein said RNA complex comprises a plurality of double-stranded segments each of which has substantial sequence identity to a target polynucleotide sequence.

135. (New) The expression construct of claim 108, wherein at least one of said

first or second strand of said RNA complex comprises a mismatched region.

136. (New) The expression construct of claim 108, wherein said double-stranded stem region of said RNA molecule comprises a first and a second strand, wherein at least one of said first or second strand of one or more of said double-stranded stem region comprises a mismatch region.

137. (New) The expression construct of claim 136, wherein said mismatch region is present in said first and second strands of one or more of said double-stranded stem regions.

138. (New) The expression construct of claim 108, wherein at least one of said loop region of said RNA molecule comprises 4 to 10 nucleotides that do not base-pair with a nucleotide of said RNA molecule.

139. (New) The expression construct of claim 129, wherein at least one of said loop region of said RNA complex comprises 4 to 10 nucleotides that do not base-pair with a nucleotide of said RNA complex.

140. (New) The expression construct of claim 108, wherein said RNA molecule

comprises a mismatched region at the 5' or 3' end that covalently links said RNA molecule to a 3' or 5' end, respectively, of a second RNA molecule.

141. (New) The expression construct of claim 108, wherein a plurality of said RNA molecules are covalently linked in a 5' to 3' orientation.

142. (New) The expression construct of claim 108, wherein said target polynucleotide sequence is selected from the group consisting of a host gene, a gene of a pathogen, or a reporter gene.

143. (New) The expression construct of claim 142, wherein said host gene encodes a polypeptide associated with a biological activity of a cell.

144. (New) The expression construct of claim 143, wherein said polypeptide is associated with a cancer, abnormal cell growth, a disease or disorder, or double-stranded ribonucleic acid (dsRNA)-mediated toxicity.

145. (New) The expression construct of claim 144, wherein said disease or disorder is an autosomal dominant or recessive disorder.

146. (New) The expression construct of claim 142, wherein said gene of a pathogen encodes a polypeptide associated with a biological activity of a pathogen.

147. (New) The expression construct of claim 146, wherein said polypeptide is associated with infection, propagation, or pathogenesis of said pathogen in a host animal.

148. (New) The expression construct of claim 142, wherein said RNA complex or RNA molecule comprises double-stranded segments having substantial sequence identity to a gene from two or more pathogens.

149. (New) The expression construct of claim 143, wherein said polypeptide is a cellular receptor that mediates infection of a cell of said host animal by said pathogen

150. (New) The expression construct of claim 142, wherein said pathogen is selected from the group consisting of a virus, a bacterium, a yeast, a protozoan, a fungus, and a parasite.

151. (New) The expression construct of claim 108, wherein said target polynucleotide sequence is in a cell of a host animal.

152. (New) The expression construct of claim 151, wherein said host animal is a mammal.

153. (New) The expression construct of claim 152, wherein said mammal is a human.

154. (New) The expression construct of claim 108, wherein said expression construct is a plasmid.

155. (New) The expression construct of claim 108, wherein said expression construct further comprises one or more of the following:

a promoter, a 5' initiation sequence, a 3' termination sequence, a sequence encoding a 5' hairpin, a sequence encoding a constitutive transport element (CTE) sequence, a sequence encoding an intron sequence, an origin of replication, a sequence encoding a polyadenylation sequence, a sequence encoding a polymerase, or a sequence encoding a selectable marker.

156. (New) The expression construct of claim 155, wherein said promoter is selected from the group consisting of an RNA Pol I promoter, an RNA Pol II promoter, an RNA Pol III promoter, and a mitochondrial promoter.

157. (New) The expression construct of claim 156, wherein said expression construct comprises at least two promoters.

158. (New) The expression construct of claim 155, wherein said promoter is selected from the group consisting of: the HCMV promoter, the T7 promoter, the Sp6 promoter, the U6 promoter, the RSV promoter, a human mitochondrial light chain promoter, and a human mitochondrial heavy chain promoter.

159. (New) The expression construct of claim 155, wherein said expression construct further encodes an RNA polymerase.

160. (New) The expression construct of claim 159, wherein said polymerase is selected from a T7 RNA polymerase, an SP6 RNA polymerase, and a ribonucleic acid (RNA)-dependent RNA polymerase.

161. (New) The expression construct of claim 108, wherein said expression construct further comprises about 1 to 150 nucleotides that flank the 5' or 3' end of said region encoding said RNA complex or said RNA molecule.

162. (New) The expression construct of claim 161, wherein at least one of the nucleotides that flank said region is a purine and said purine is the 5' nucleotide of the polynucleotide sequence.

163. (New) The expression construct of claim 161, wherein said expression construct comprises four guanosine bases at the 5' of said region encoding said RNA complex or said RNA molecule.

164. (New) A pharmaceutical composition comprising the expression construct of claim 108, wherein said pharmaceutical composition includes a physiologically acceptable excipient.

165. (New) The pharmaceutical composition of claim 164, wherein said expression construct is provided as a plasmid.

166. (New) The pharmaceutical composition of claim 165 further comprising a polynucleotide transfection facilitating agent.

167. (New) A pharmaceutical composition comprising the RNA complex or the RNA molecule encoded by the expression construct of claim 108, wherein said

pharmaceutical composition includes a physiologically acceptable excipient.

168. (New) The pharmaceutical composition of claim 165 further comprising a polynucleotide transfection facilitating agent.

169. (New) A method for generating a ribonucleic acid (RNA) complex comprising a first strand and a second strand that hybridize to each other under physiological conditions to form a first double-stranded region, said first double-stranded region comprising one or more mismatched regions that separate said first double-stranded region into two or more double-stranded segments, wherein said mismatched regions are susceptible to cleavage by single-strand ribonucleases and wherein at least a portion of at least one of said double-stranded segments has substantial sequence identity to a target polynucleotide sequence, or

an RNA molecule comprising one or more stem-loop structures comprising a double-stranded stem region and a single-stranded loop region each separated by a single-stranded spacer region, wherein the double-stranded stem region of at least one stem-loop structure comprises one or more mismatched regions, wherein said one or more mismatched regions separates said double stranded stem region into two or more double stranded segments, wherein said mismatched regions are susceptible to cleavage by single-strand ribonucleases and wherein at least a portion of at least one of said double-

stranded segments has substantial sequence identity to a target polynucleotide sequence, wherein said method comprises contacting an expression construct encoding said RNA complex or RNA molecule with cell-free components or by administering the expression construct to a cell or a mammal, under conditions that allow expression of said RNA complex or RNA molecule.

170. (New) The method of claim 169, wherein said target polynucleotide sequence is selected from the group consisting of a host gene, a gene of a pathogen, or a reporter gene.

171. (New) A method for reducing or inhibiting the expression of a target gene in a cell, said method comprising administering to a subject in need thereof a nucleic acid molecule that comprises or that encodes

a ribonucleic acid (RNA) complex comprising a first strand and a second strand that is capable of hybridizing to each other or that has hybridized to each other under physiological conditions to form a first double-stranded region, said first double-stranded region comprising one or more mismatched regions that separate said first double-stranded region into two or more double-stranded segments, wherein said mismatched regions are susceptible to cleavage by single-strand ribonucleases and wherein at least a portion of at least one of said double-stranded segments has substantial sequence identity

to a target polynucleotide sequence, or

an RNA molecule comprising one or more stem-loop structures comprising a double-stranded stem region and a single-stranded loop region each separated by a single-stranded spacer region, wherein the double-stranded stem region of at least one stem-loop structure comprises one or more mismatched regions, wherein said one or more mismatched regions separates said double stranded stem region into two or more double stranded segments, wherein said mismatched regions are susceptible to cleavage by single-strand ribonucleases and wherein at least a portion of at least one of said double-stranded segments has substantial sequence identity to a target polynucleotide sequence,

wherein said administering occurs under conditions that allow cleavage of said expressed or administered RNA complex or RNA molecule by a single-stranded RNA-specific ribonuclease (RNase) to liberate said double-stranded segments of said RNA complex or RNA molecule, wherein said liberated double-stranded segments from said RNA complex or RNA molecule are capable of reducing or inhibiting the expression of a target polynucleotide sequence, relative to the expression of said target polynucleotide sequence in a cell lacking said RNA complex or RNA molecule.

172. (New) The method of claim 171, wherein said target polynucleotide sequence is selected from the group consisting of a host gene, a gene of a pathogen, or a reporter gene.

173. (New) An RNA complex comprising a first strand and a second strand that are capable of hybridizing to each other under physiological conditions to form a first double-stranded region, said first double-stranded region comprising one or more mismatched regions that separate said first double-stranded region into two or more double-stranded segments, wherein said mismatched regions are susceptible to cleavage by single-strand ribonucleases and wherein at least a portion of at least one of said double-stranded segments has substantial sequence identity to a target polynucleotide sequence.

174. (New) An RNA molecule comprising one or more stem-loop structures comprising a double-stranded stem region and a single-stranded loop region each separated by a single-stranded spacer region, wherein the double-stranded stem region of at least one stem-loop structure comprises one or more mismatched regions, wherein said one or more mismatched regions separates said double stranded stem region into two or more double stranded segments, wherein said mismatched regions are susceptible to cleavage by single-strand ribonucleases and wherein at least a portion of at least one of said double-stranded segments has substantial sequence identity to a target polynucleotide sequence.